

Figure 1: RNA Amplification Method

ROUND ONE:

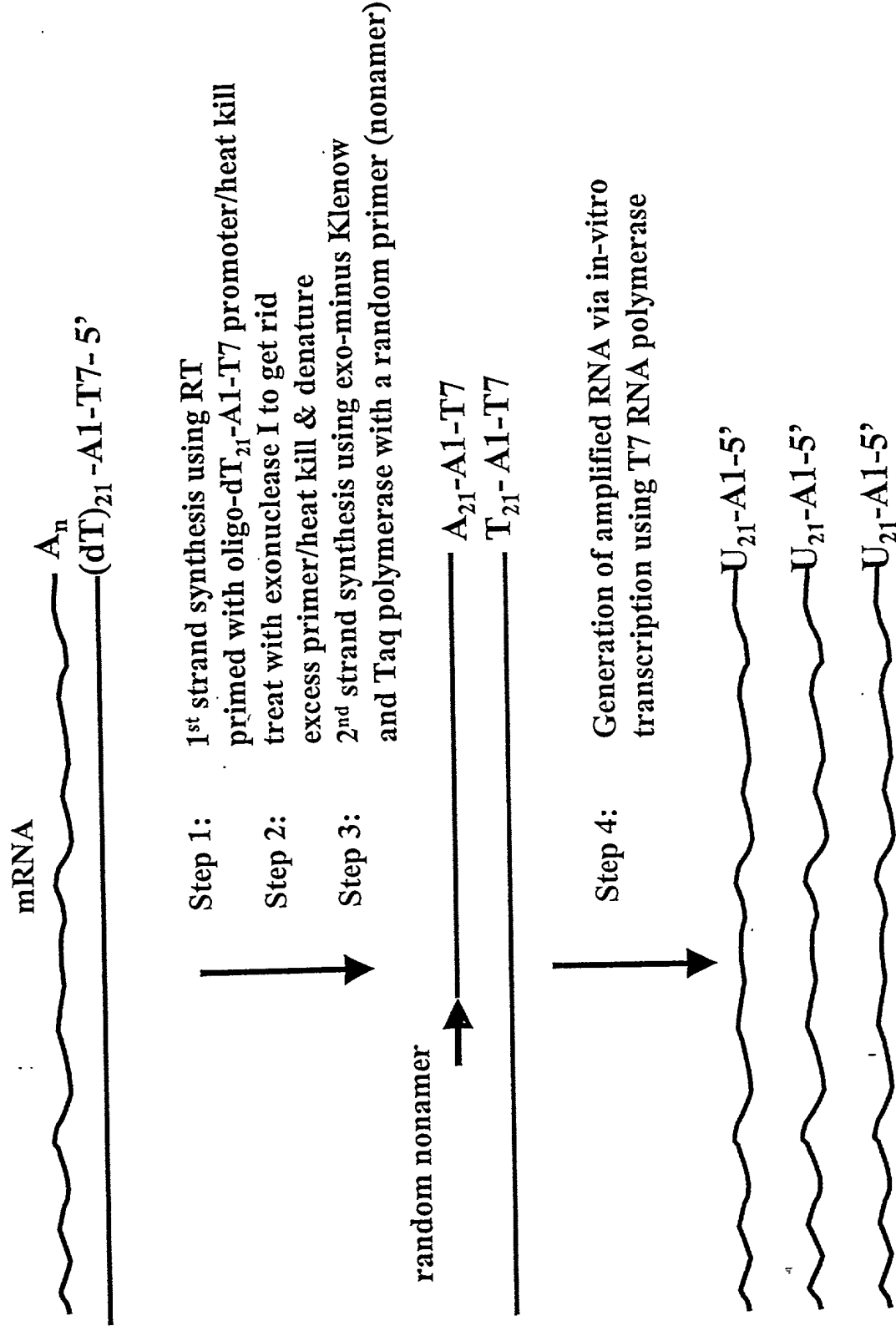


Figure 1: RNA Amplification Method cont.

ROUND TWO:

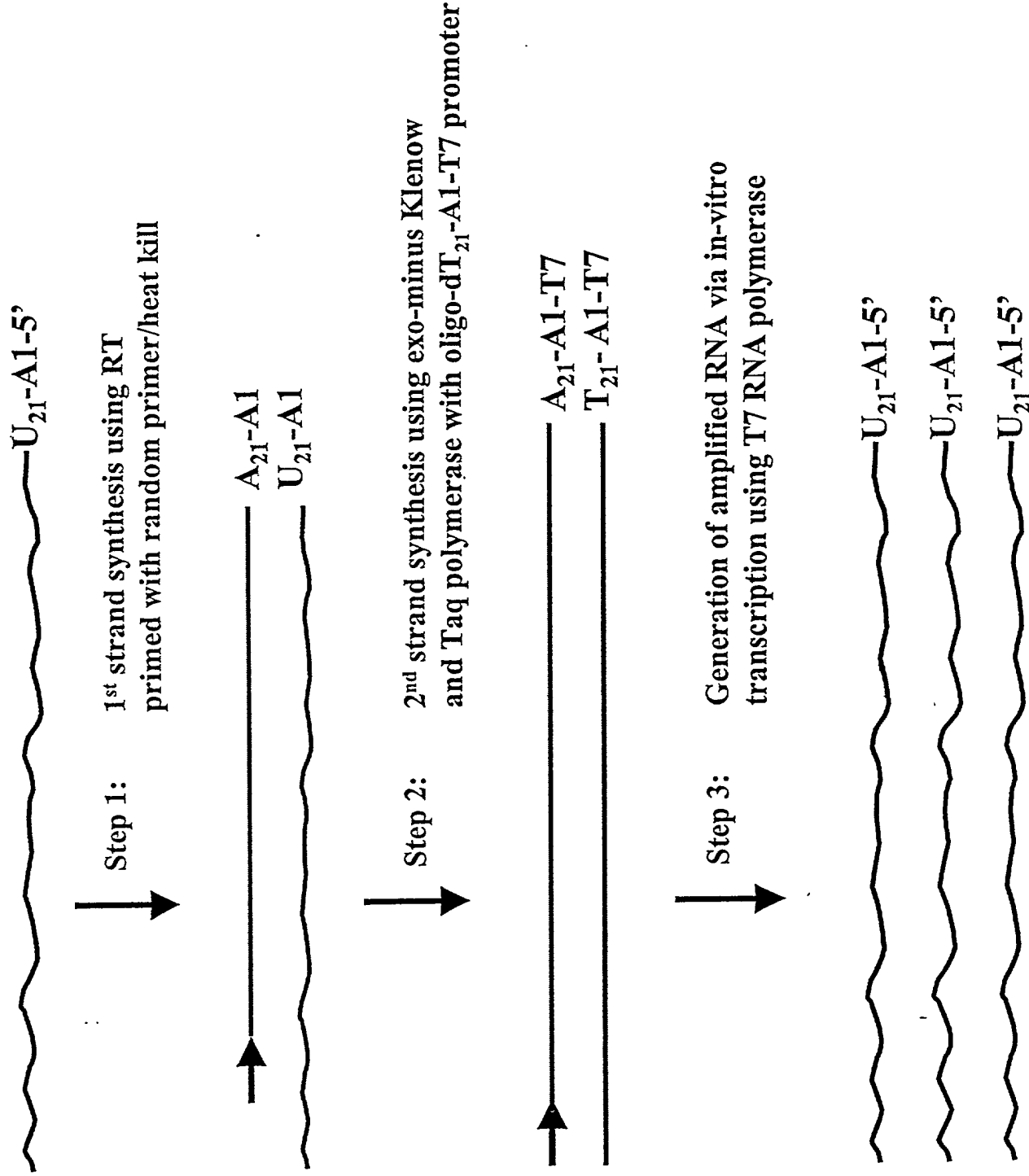


Figure 1: RNA Amplification Method cont.

ROUND TWO MODIFIED:

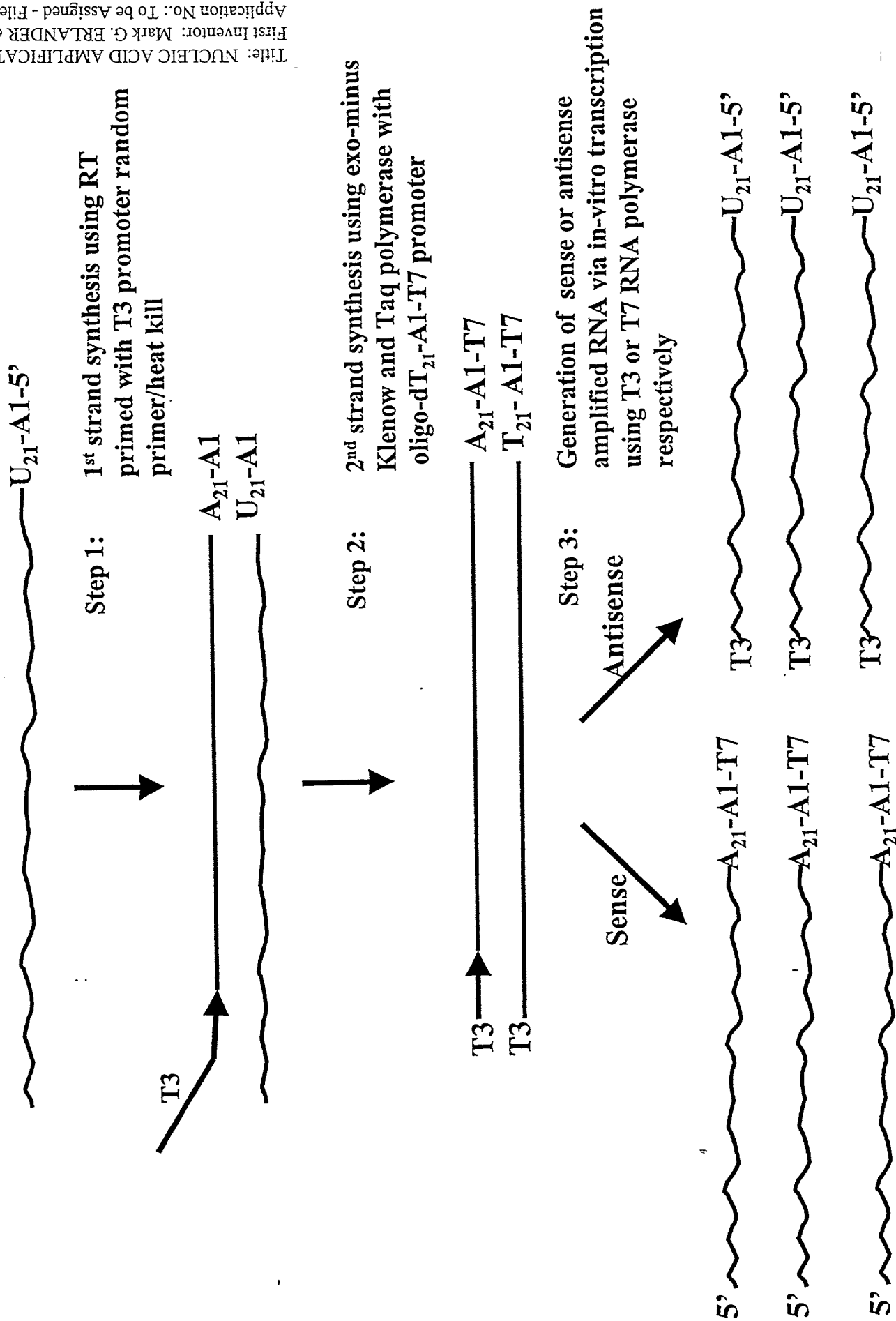
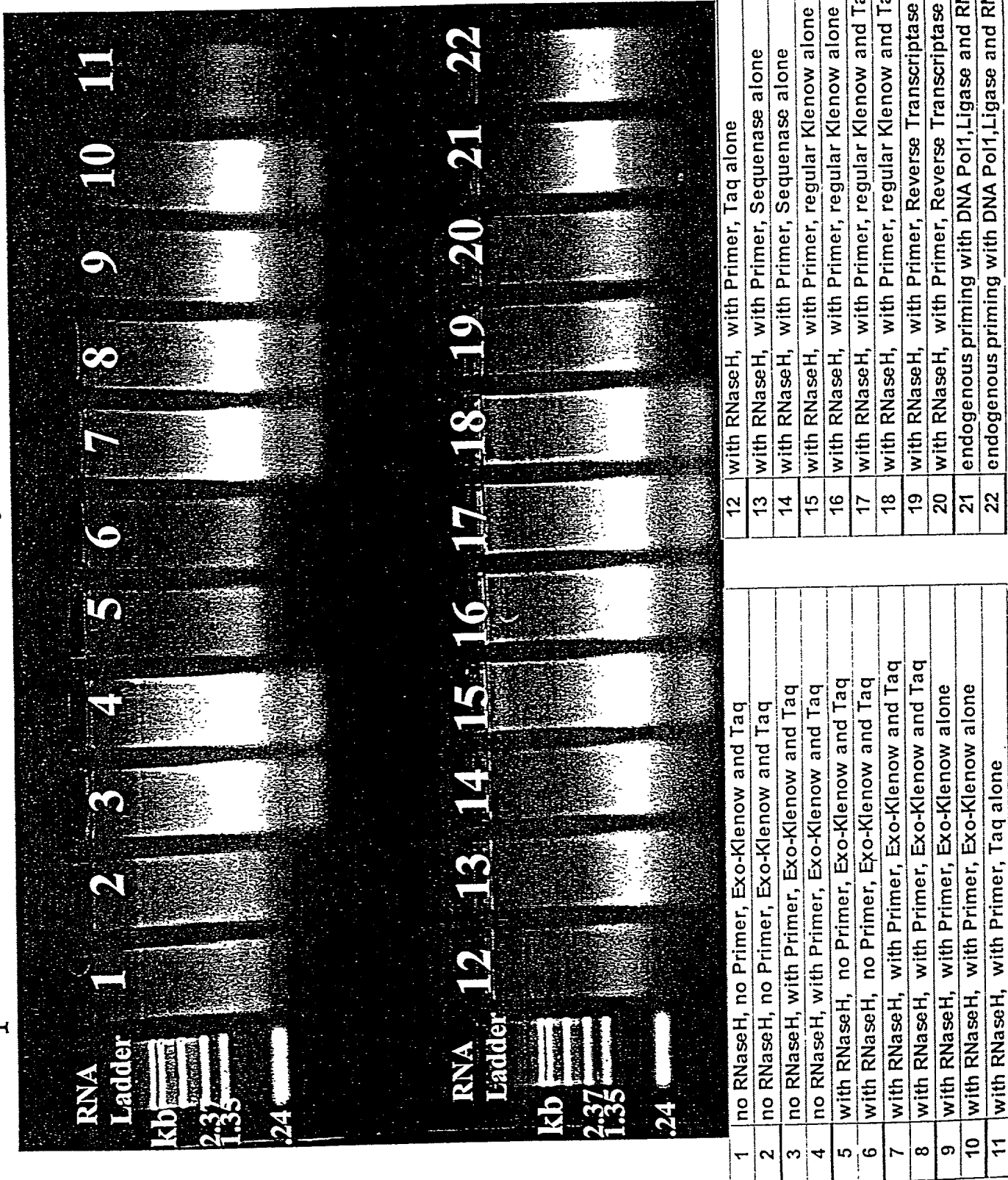


Figure 2A: Optimization of 2nd Strand Synthesis using Exogenous Primers



FOSSOT 4333001

Figure 2B: Yields From Exogenous Priming of 2nd Strand Synthesis Using Different Enzymes

SAMPLES	Condition Tested	ug of amplified RNA
1	no RNaseH, no Primer, Exo-Klenow and Taq	3.6
2		3.4
3	no RNaseH, with Primer, Exo-Klenow and Taq	15.5
4		19.2
5	with RNaseH, no Primer, Exo-Klenow and Taq	3.4
6		3.0
7	with RNaseH, with Primer, Exo-Klenow and Taq	16.9
8		17.5
9	with RNaseH, with Primer, Exo-Klenow alone	18.7
10		16.8
11	with RNaseH, with Primer, Taq alone	2.8
12		3.6
13	with RNaseH, with Primer, Sequenase alone	9.0
14		10.4
15	with RNaseH, with Primer, regular Klenow alone	16.0
16		15.2
17	with RNaseH, with Primer, regular Klenow and Taq	13.7
18		15.2
19	with RNaseH, with Primer, Reverse Transcriptase alone	7.2
20		6.5
21 Eberwine1	endogenous priming method with DNA Pol1, Ligase and RNaseH	10.2
22 Eberwine2		11.7

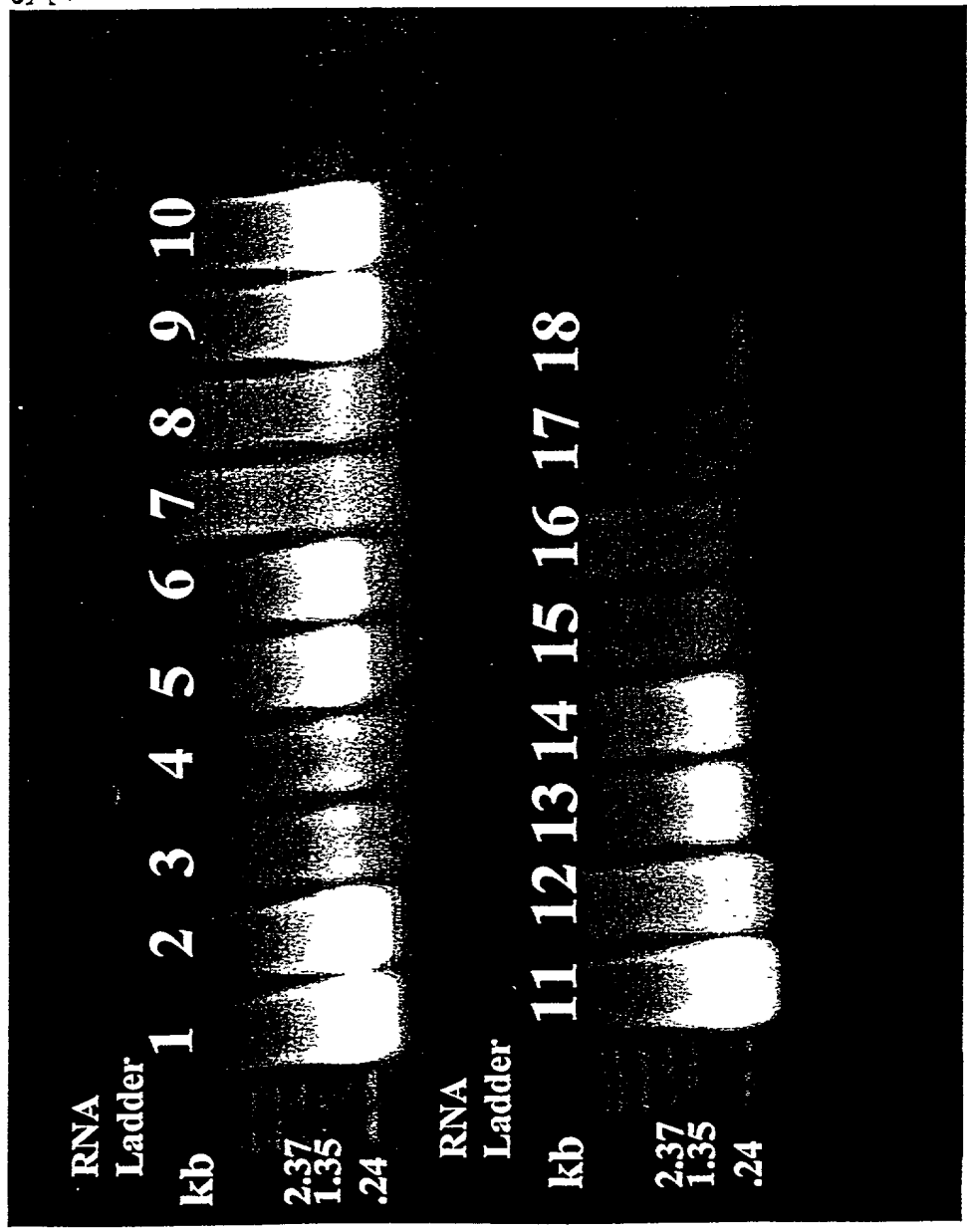
Figure 2C: Comparison of Yields and Fold Amplification

SAMPLES	Condition Tested	ave (ug)	fold diff vs GH	est. fold amp*
1	no RNaseH, no Primer, Exo-Klenow and Taq	3.5	0.3	174
2				
3	no RNaseH, with Primer, Exo-Klenow and Taq	17.3	1.6	865
4				
5	with RNaseH, no Primer, Exo-Klenow and Taq	3.2	0.3	159
6				
7	with RNaseH, with Primer, Exo-Klenow and Taq	17.2	1.6	862
8				
9	with RNaseH, with Primer, Exo-Klenow alone	17.7	1.6	887
10				
11	with RNaseH, with Primer, Taq alone	3.2	0.3	161
12				
13	with RNaseH, with Primer, Sequenase alone	9.7	0.9	486
14				
15	with RNaseH, with Primer, regular Klenow alone	15.6	1.4	778
16				
17	with RNaseH, with Primer, regular Klenow and Taq	14.4	1.3	721
18				
19	with RNaseH, with Primer, Reverse Transcriptase alone	6.8	0.6	342
20				
21 Eberwine1	endogenous priming method with DNA Pol1, Ligase and RNaseH	11.0	1.0	548
22 Eberwine2				

*fold-amplification calculated as follows: (final μg yield)/(0.020 μg)
 where 0.020 μg is an estimate based on the assumption that 2% of 1 μg
 of total RNA (the amount of starting material) is poly(A) RNA

FIGURE 3A

Figure 3A: mRNAs can be amplified from nanogram amounts of total RNA



Starting Total RNA	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
10 ng	Eberwine Method	Eberwine Method	Eberwine Method	Eberwine Method	Exo-klenow/ Taq with T7A1	Exo-klenow/ Taq with T7A1	Exo-klenow/ Taq with T7A1	Exo-klenow/ Taq with T7A1	Exo-klenow/ Taq with T7A1dT	Exo-klenow/ Taq with T7A1dT	Exo-klenow/ Taq with T7A1dT	Exo-klenow/ Taq with T7A1dT	Regular klenow/Taq with T7A1	Regular klenow/Taq with T7A1	Regular klenow/Taq with T7A1	Regular klenow/Taq with T7A1	negative control--no RNA	negative control--no RNA

Figure 3B: Quantitation of amplified RNA

Total RNA			conc (ng/ml)	yield
10 ng	1	Eberwine method	1860	101.0
10 ng	2	Eberwine method	1800	97.4
2 ng	3	Eberwine method	448	26.9
2 ng	4	Eberwine method	439	26.3
10 ng	5	exo-klenow + taq with t7a1	946	46.2
10 ng	6	exo-klenow + taq with t7a1	945	46.1
2 ng	7	exo-klenow + taq with t7a1	518	20.5
2 ng	8	exo-klenow + taq with t7a1	464	17.2
10 ng	9	exo-klenow + taq with t7a1dt	1700	91.4
10 ng	10	exo-klenow + taq with t7a1dt	1825	98.9
2 ng	11	exo-klenow + taq with t7a1dt	2400	144.0
2 ng	12	exo-klenow + taq with t7a1dt	648	38.9
10 ng	13	regular klenow + taq with t7a1	780	36.2
10 ng	14	regular klenow + taq with t7a1	808	37.9
2 ng	15	regular klenow + taq with t7a1	313	8.2
2 ng	16	regular klenow + taq with t7a1	298	7.3